

Abstract

Solid Phase Sequencing

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The present invention describes methods of sequencing a nucleic acid in a sample, based on the use of terminal-phosphate-labeled nucleotides as substrates for nucleic acid polymerases. The methods provided by this invention utilize a nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogue which has a colorimetric dye, chemiluminescent, or
10 fluorescent moiety, a mass tag or an electrochemical tag attached to the terminal-phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label would be present on the inorganic polyphosphate by-product of phosphoryl transfer. Cleavage of the polyphosphate product of phosphoryl transfer
15 *via* phosphatase leads to a detectable change in the label attached thereon. In some instances the labeled polyphosphate may be detected directly via the label and provide information on the nucleic acid. When the polymerase assay is performed in the presence of a phosphatase, there is provided a convenient method for real-time monitoring of DNA or RNA synthesis and characterization of a target nucleic acid.

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